SUPPLEMENTARY INFORMATION

Figure S1. Schematic showing genomic incorporation of mutant *arnA* into the *arnA* deletion strain using a modified Recombineering approach

Mutant *arnA* and the selectable marker were PCR amplified separately. Using a designed overlap region at the 5' and 3' ends of the *arnA* mutant and the selectable marker, respectively, the two PCR products were subsequently combined in a third PCR reaction as shown. The final PCR product was transformed into the *arnA* deletion strain and integrated genomically according to the original recombineering protocol[10]. Primers used for each reaction are indicated in boxes and primer sequences can be found in Table S1.

Figure S2. Structures of *E. coli* ArnA and SlyD

A. The crystal structure of hexameric *E. coli* ArnA shown in surface representation comparing the surface-exposed histidine residues in wild type versus mutant ArnA. ArnA

is a dimer of trimers, with the subunits of the top trimer shown in gray and the bottom trimer shown in white. Histidine residues are colored yellow, except for those that were mutated to serines, which are highlighted in red. Black lines indicate the trimer interfaces. **B**. NMR structure of *E. coli* SlyD shown in cartoon representation with histidine residues colored red. The arrow indicates where SlyD was truncated to remove the histidine rich C-terminal tail.

Figure S3. Mutant ArnA has reduced Ni-binding affinity compared to wild type ArnA

Both mutant and wild type ArnA were expressed and purified recombinantly, loaded onto a His-Trap column, and eluted with a linear gradient from 0-150 mM imidazole. The elution profiles for wild type and mutant ArnA are shown in black and red, respectively. The imidazole gradient is shown by a dashed black line and the imidazole concentrations at which the proteins eluted are indicated. The recombinant ArnA mutant has significantly reduced Ni-affinity compared to recombinant wild type ArnA.